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<p>(54) Title: INJECTABLE FORMULATIONS FOR TREATMENT OF OSTEOPOROTIC BONE</p> <p>(57) Abstract</p> <p>Methods and compositions are provided for the treatment of defects and disease involving osteoporosis, or osteopenic conditions. The methods comprise applying to the site of osteoporotic or osteopenic conditions a composition comprising an active agent, preferably from the TGF-β superfamily of proteins. The advantages of the invention include a reduction or avoidance of the severity and/or incidence of bone fractures. Also, the methods of the present invention are advantageous in that administration is local, rather than systemic.</p>			

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INJECTABLE FORMULATIONS FOR TREATMENT OF OSTEOPOROTIC BONE

FIELD OF THE INVENTION

5 The present invention relates to the field of tissue repair, specifically, to the treatment of osteoporotic bone and/or prevention of osteoporosis. Osteoporotic or osteopenic bone is often characterized by suboptimal bone density. The osteoporotic condition may be related to diet, trauma or stress, or to degenerative or congenital disease. Thus, the present invention may be useful in the treatment and/or the 10 prevention of osteoporosis, or the treatment of osteoporotic or osteopenic bone.

BACKGROUND OF THE INVENTION

Background of the incidence and etiology of need: Idiopathic osteoporosis is a disease of unknown etiology characterized by progressive loss of bone mass and increased fragility. It is a medical problem because it is associated with a marked 15 increase in susceptibility to fractures. It is a public health problem for several reasons. First, it is among the most prevalent of all musculoskeletal disorders. Fifty-six percent of women over 45 years of age are afflicted. Praemer *et al.*, *Musculoskeletal Conditions in the United States*, American Academy of Orthopaedic Surgeons, Park Ridge, IL (1992). Second, its incidence increases with age. Because the percentage 20 of elderly in the population is increasing, osteoporosis will become more common with time. Third, osteoporosis presently has no known cure, and is difficult to treat locally. Fourth, it is a major economic burden to individuals and to society. Fifth, and most significantly, osteoporosis is associated with a substantial morbidity and mortality. The most serious fracture resulting from osteoporosis is that of the 25 proximal femur in the region of the hip joint. With an annual incidence of 250,000, hip fractures are currently the most common fracture in the elderly. Praemer *et al.*, *Ibid.* Estimates based on census projections indicate that this figure will increase to approximately 340,000 hip fractures per year by the year 2000. One out of every six caucasian women will have a hip fracture during her lifetime (Cummings *et al.*, *Arch Intern Med* 149:2455-2458 (1989), and for those who attain the age of 90, this figure 30 becomes one in three.

5 Of the patients who are independent and living at home at the time of hip fracture, approximately 20 percent remain in a long term care institution for at least one year following the fracture. During the first year following injury, the mortality rate is approximately 15% higher than for age and gender matched controls. Praemer *et al.*, *Ibid.* The financial toll of these fractures is also high. In 1988, hip fractures 10 cost the United States 8.7 billion dollars. Praemer *et al.*, *Ibid.* The increased incidence of proximal femur fracture observed in elderly patients is mainly related to two factors: (1) a decreased bone density of their proximal femora; (2) an increased propensity to fall. There is an inverse relationship between the age-related bone loss 15 in the proximal femur and the risk of hip fracture. Each decrease of one standard deviation (SD) in femoral neck bone density increases the age-adjusted risk of hip fracture 2.6 times (95% CI 1.9 - 3.6); and (3) women with bone density in the lowest quartile have an 8.5-fold greater risk of hip fracture than those in the highest quartile. Cummings *et al.*, *The Lancet* 341:72-75 (1993). This relation between hip bone mass 20 and hip fracture risk allows the screening and identification of patients at risk for fracture. Patients who are two standard deviations below peak hip bone mass have passed beneath the "fracture threshold."

25 Bioactive agents that reduce the incidence of hip fractures or augment the healing of such fractures when they occur would substantially improve the health of the elderly. The availability of an injectable agent would permit its use in fracture prevention, such as hip fracture, without the costs and morbidity of surgical intervention, such as for femoral neck fractures. Such an agent would have application in fracture treatment, without increasing the extent or complexity of the surgical management. The inventors have provided a novel method using bioactive 30 agents to decrease the occurrence and/or severity of fractures to osteoporotic bone, such as an osteoporotic hip.

35 While several therapies for osteoporosis have been approved, there is none that addresses the localized treatment of proximal femur osteoporosis. Current therapies for osteoporosis are systemic. These include fluoride, bisphosphonates, calcitonin, estrogens and progestins, testosterone, vitamin D metabolites, and/or calcium. In the United States, only estrogens and alendronate, a bisphosphonate, are indicated for the

5 prevention of hip fractures in postmenopausal osteoporotic women. Each of these
agents requires continuous administration over a time period of years. Estrogen has
undesirable side effects (WHO Study Group on Assessment of Fracture Risk and its
Application to Screening for Postmenopausal Osteoporosis. WHO Technical Report
Series 843, Assessment of fracture risk and its application to screening for
10 postmenopausal osteoporosis. Geneva, World Health Organization (1992)),
compliance is poor, and it is not indicated in males. Alendronate requires carefully
separated administration of the drug and sources of calcium and is not recommended
in patients with reduced renal function. A recent study found a reduction in hip
fractures in elderly women with involutional osteoporosis after eighteen months of
15 vitamin D and calcium (Chapuy *et al.* New Engl J. Med. 327:1637-1642 (1992)), but
this treatment has not been conformed by independent sources, and lacks United
States regulatory approval. While several treatments have effectively arrested or
decreased bone loss, only the use of fluoride has been associated with rapid increases
in bone density. However, fluoride treatment increased bone density less in the
20 femoral region as compared to the spine and was associated with poor bone quality.
Hedlund *et al.*, J. Bone Min. Res. 4:223-225 (1989). Fluoride treatment again has not
received regulatory approval. Thus, no safe, effective, generally applicable local
treatment is available which protects against the occurrence of hip fractures in
osteoporosis by providing a reliable and durable increase in hip bone mass.
25 Accordingly, despite substantial endeavors in this field, there remains a need for an
effective method of repair and/or treatment of osteoporotic and osteopenic bone, and
for treatment and/or prevention of osteoporosis.

SUMMARY OF THE INVENTION

30 Accordingly, the present invention provides methods and compositions for
increasing bone mass and quality, and for minimizing or reducing the incidence or
severity of osteoporosis-related fractures. Accordingly, the present invention provides
methods and compositions useful for decreasing the incidence of fractures of
osteoporotic or osteopenic bone. In particular, the present invention comprises
35 methods of treating patients with osteoporosis, or with other evidence of osteoporosis

5 or osteopenic condition. Preferred embodiments where the present invention may prove particularly useful include treatment of metaphyseal bone, including proximal femur (hip), proximal humerus (upper arm), distal radius (wrist), and vertebral bodies (spine), particularly the vertebral body.

10 The method comprises administering to a site of osteopenic or osteoporotic bone, or a site of low bone mass or density, an effective amount of a composition comprising at least one active agent which is capable of inducing growth of bone or increasing the formation of bone tissue or reducing bone loss at the site. Bone mass is commonly designated "bone mineral content" or BMC and is measured in grams. Bone density is commonly designated "bone mineral density" or BMD and is expressed as grams per unit area or grams per unit volume. In a preferred embodiment, the mode of administration is by intraosseous injection. In preferred embodiments, the active agent is one or more proteins selected from the group of proteins known as the Transforming Growth Factors-Beta (TGF- β) superfamily of proteins, preferably selected from the Bone Morphogenetic Proteins (BMPs), the Growth and Differentiation Factors (GDFs), as well as other proteins, as described more fully herein. The methods and compositions of the present invention are advantageous in that they provide a localized treatment for osteoporosis or osteopenic bone, rather than systemic treatment. The present invention is further advantageous in that it utilizes as active agents osteogenic proteins, which may be produced via recombinant DNA technology, and therefore are of potentially unlimited supply. The methods and compositions of the present invention are further advantageous in that regeneration of the bone tissue increases the bone mass/density, increase the bone strength, and thereby reduce the severity of osteoporosis or incidence of osteoporotic lesions, ultimately lessening the incidence of bone fractures.

30 In a preferred embodiment of the present invention, the active agents are administered locally through injection using a suitable buffer and/or carrier. One suitable buffer comprises glycine, sucrose, and glutamic acid hydrochloride, at a pH of less than 6.0. In a preferred embodiment of the invention, this formulation comprises about 2.5% glycine (g/100 ml (w/v)), about 0.5% sucrose (w/v), about 5 mM glutamic acid hydrochloride (about 0.1% w/v), and about 0.01% (w/v)

5 polysorbate 80, at a pH of about 4.5. This buffer is referred to later in this application
as "MFR 842." Further buffers suitable for use in the present invention are described
in United States Patent 5,385,887, the disclosure of which is hereby incorporated by
reference. Suitable carriers include collagen gels, hyaluronate, alginates and
10 hyaluronic acids, injectable calcium phosphates, polyols, demineralized bone matrix
and combinations of the above. Other carriers which may be useful for the present
invention include blood as well as clotting proteins, such as fibrin or thrombin, and
oils.

15 In other preferred embodiments, the active agent further comprises, in addition
to one or more proteins selected from the TGF- β superfamily of proteins, one or more
auxiliary proteins, such as *Hedgehog*, *Noggin*, *Chordin*, *Frazzled*, *Cerberus* and
20 *Follistatin*, soluble BMP receptors, or other protein or agent, as described further
herein.

25 In addition to healing of osteoporotic bone, compositions of the present
invention may be useful for injectable formulations of BMPs for uses such as injection
into joints for treatment and repair of osseous defects, cartilage defects, inhibition of
cartilage degradation and to promote cartilage repair. The formulations may also be
injected into tendons, ligaments and/or their attachment sites to bone. Injectable
formulations of BMPs may also find application to other bone sites such as bone cysts,
implants into bones, closed fractures and distraction osteogenesis.

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DETAILED DESCRIPTION OF THE INVENTION

According to the present invention, methods and compositions are provided
30 for treatment of patients who exhibit signs of osteoporosis, or osteopenic conditions,
including osteoporotic bone lesions. The identification of such patients may be
accomplished by procedures which are well known in the art. These procedures
include measurement of bone mass/density using dual-energy X-ray absorptiometry
35 (DEXA), Kilgus et al., J. Bone & Joint Surgery, 75-B:279-287 (1992); Markel et al.,
Acta Orthop Scand, 61:487-498 (1990); and quantitative computed tomography
(QCT), Laval-Jeantet et al., J Comput Assist Tomogr, 17:915-921 (1993); Markel,
35 Calcif Tissue Int, 49:427-432 (1991); single-photon absorptiometry, Markel et al.

5 Calcif Tissue Int, 48:392-399 (1991); ultrasound transmission velocity (UTV); Heaney et al., JAMA, 261:2986-2990 (1989); Langton et al., Clin Phys Physiol Meas, 11:243-249 (1990); and radiographic assessment, Gluer et al., J Bone & Mineral Res, 9:671-677 (1994). Other methods of identification of patients at risk of bone fracture include assessment of age-related factors, such as cognisance, as well as prior occurrence of
10 osteoporosis-related fractures. Porter et al., BMJ, 301: 638-641 (1990); Hui et al., J Clin Invest, 81:1804-1809 (1988). The above publications are hereby incorporated by reference herein.

15 The methods comprise applying to the osteoporotic or osteopenic site an amount of a composition comprising one or more purified osteogenic proteins which is effective to induce the formation and/or maintenance of bone.

ACTIVE AGENT

20 The active agent is preferably selected from the family of proteins known as the transforming growth factors-beta (TGF- β) superfamily of proteins, which includes the activins, inhibins and bone morphogenetic proteins (BMPs). Most preferably, the active agent includes at least one protein selected from the subclass of proteins known generally as BMPs, which have been disclosed to have osteogenic activity, and other growth and differentiation type activities. These BMPs include BMP proteins BMP-2, BMP-3, BMP-4, BMP-5, BMP-6 and BMP-7, disclosed for instance in United States Patents 5,108,922; 5,013,649; 5,116,738; 5,106,748; 5,187,076; and 5,141,905; BMP-25 8, disclosed in PCT publication WO91/18098; and BMP-9, disclosed in PCT publication WO93/00432, BMP-10, disclosed in PCT application WO94/26893; BMP-11, disclosed in PCT application WO94/26892, or BMP-12 or BMP-13, disclosed in PCT application WO 95/16035; BMP-15, disclosed in United States Patent 5,635,372; or BMP-16, disclosed in co-pending patent application, serial no. 08/715,202, filed on September 18, 1996. Other TGF- β proteins which may be useful as the active agent in the present invention include Vgr-2, Jones et al., Mol. Endocrinol., 6:1961-1968 (1992), and any of the growth and differentiation factors [GDFs], including those described in PCT applications WO94/15965; WO94/15949; WO95/01801; WO95/01802; WO94/21681; WO94/15966; WO95/10539;
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5 WO96/01845; WO96/02559 and others. Also useful in the present invention may be
BIP, disclosed in WO94/01557; HP00269, disclosed in JP Publication number:
7-250688; and MP52, disclosed in PCT application WO93/16099. The disclosures
of all of the above applications are hereby incorporated by reference. A subset of
BMPs which are presently preferred for use in the present invention include BMP-2,
10 BMP-4, BMP-5, BMP-6, BMP-7, BMP-10, BMP-12 and BMP-13. The active agent
is most preferably BMP-2, the sequence of which is disclosed in United States Patent
5,013,649, the disclosure of which is hereby incorporated by reference. Other BMPs
and TGF- β proteins known in the art can also be used.

15 The active agent may be recombinantly produced, or purified from a protein
composition. The active agent, if a TGF- β such as a BMP, or other dimeric protein,
may be homodimeric, or may be heterodimeric with other BMPs (e.g., a heterodimer
composed of one monomer each of BMP-2 and BMP-6) or with other members of the
TGF- β superfamily, such as activins, inhibins and TGF- β 1 (e.g., a heterodimer
composed of one monomer each of a BMP and a related member of the TGF- β
20 superfamily). Examples of such heterodimeric proteins are described for example in
Published PCT Patent Application WO 93/09229, the specification of which is hereby
incorporated herein by reference.

25 The active agent may further comprise additional agents such as the *Hedgehog*,
Frazzled, *Chordin*, *Noggin*, *Cerberus* and *Follistatin* proteins. These families of
proteins are generally described in Sasai et al., Cell, 79:779-790 (1994) (*Chordin*);
PCT Patent Publication WO94/05800 (*Noggin*); and Fukui et al., Developmental
Biology, 159:131-139 (1993) (*Follistatin*). *Hedgehog* proteins are described in
WO96/16668; WO96/17924; and WO95/18856. The *Frazzled* family of proteins is
30 a recently discovered family of proteins with high homology to the extracellular
binding domain of the receptor protein family known as *Frizzled*. The *Frizzled* family
of genes and proteins is described in Wang et al., J. Biol. Chem., 271:4468-4476
(1996). The active agent may also include other soluble receptors, such as the
truncated soluble receptors disclosed in PCT patent publication WO95/07982. From
the teaching of WO95/07982, one skilled in the art will recognize that truncated
35 soluble receptors can be prepared for numerous other receptor proteins. Such would

5 also be encompassed within the present invention. The above publications are hereby incorporated by reference herein.

10 The amount of active agent useful herein is that amount effective to stimulate increased osteogenic activity of present or infiltrating progenitor or other cells, and will depend upon the size and nature of the defect being treated, as well as the carrier being employed. Generally, the amount of protein to be delivered is in a range of from about 0.1 to about 100 mg; preferably about 1 to about 100 mg; most preferably about 10 to about 80 mg.

CARRIER

15 Materials which may be useful as the carrier in practicing the present invention include pharmaceutically acceptable materials having viscosity and polarity such that, when added to the bone morphogenetic protein, form a composition that possesses appropriate handling characteristics for injectable application to the site of osteoporotic or osteopenic bone. Adding the carrier to the bone morphogenetic protein allows the protein to remain in the diseased or lesioned site for a time sufficient to allow the protein to increase the otherwise natural rate of regenerative 20 osteogenic activity of the infiltrating mammalian progenitor or other cells, and to form a space in which new tissue can grow and allow for ingrowth of cells. The carrier may also allow the bone morphogenetic protein to be released from the disease or lesion site over a time interval appropriate for optimally increasing the rate of regenerative 25 osteogenic activity of the progenitor cells. The carrier may also supply a framework on which to induce new formation in severely osteoporotic bone.

30 The most preferred family of carriers comprises collagenous materials. These are preferably in a form suitable for injection, such as a gel. Such gels may be cross-linked or non-cross-linked. Other forms of collagen, such as dispersions or fibrillar collagen, may also be useful in the methods of the present invention. Another preferred family of carriers is cellulosic materials such as alkylcellulose, including hydroxyalkylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, and carboxymethylcellulose, the most preferred being the cationic salts of carboxymethylcellulose (CMC).

5 In the case of cellulosic carriers and collagen gels, it is preferred that the carrier be in the form of a hydrated cellulosic viscous gel. Viscosity may be increased through mechanical means, such as high agitation for a suitable period of time, followed by autoclaving, or chemically. The active agent and cellulosic carrier is preferably in a solution of suitable buffer. One preferred buffer solution is a
10 composition comprising, in addition to the active agent, about 1.0 to about 10.0% (w/v) glycine, about 0.1 to about 5.0% (w/v) of a sugar, preferably sucrose, about 1 to about 20 mM glutamic acid hydrochloride, and optionally about 0.01 to about 0.1% of a non-ionic surfactant, such as polysorbate 80. Preferred solutions are from about 1% to about 20% w/v cellulosic carrier/buffer. If desired, a salt may be added. A
15 preferred viscous gel carrier is described in Example 2 below. The amount of osteogenic protein useful with viscous gel carrier is generally in a range of from about 0.1 to about 100 mg, preferably about 1 to about 100 mg; most preferably about 10 to about 80 mg per cubic centimeter of implant material required.

20 Another class of materials of particular interest for injectable carriers are resorbable hydroxyapatites as well as minerals, ceramics and phosphates. Resorbable hydroxyapatites, for example, can be formulated at various porosities with varying resorption rates; their handling characteristics vary from hard implantable types, to gel-like consistency, to those that are injectable but harden at body temperature. Suitable hydroxyapatite and ceramic carriers are described, for example in
25 WO96/36562; and United States Patents 5,543,019; 5,306,305; 5,258,044; 5,496,399; 5,455,231; 5,336,264; 5,178,845; 5,053,212; 5,047,031; 5,129,905; 5,034,059; 4,880,610; 5,290,763; and 5,563,124; the disclosures of which are incorporated herein by reference.

30 Another preferred family of carriers for administration of the active agent of the present invention are injectable polymers, which may be viscous and which may optionally include a sequestering agent as well. Suitable polymers and sequestering agents include those described in United States Patent 5,171,579, the entire disclosure of which is incorporated herein by reference. Other polymers include the pluronic, such as Poloxamer 407 gel. Pluronics are a class of water soluble ABA type block
35 surfactant copolymers which exhibit the unique property of reverse thermal gelation.

5 They are liquid (and hence syringeable) at 4°C and gel at body temperature. Poloxamer 407, MW 12,500, is excreted unchanged in the urine after systemic absorption and has supposedly been shown to be non-toxic in animals. Polylactides and/or polyethylene glycols, including poly(lactide)/poly(ethylene glycol) gels. Polylactides may be dissolved in polyethylene glycols, such as low molecular weight
10 (2000) PLA dissolved in PEG to produce a syringeable solution that precipitates PLA upon injection into an aqueous environment, resulting in a relatively firm gel. In addition, the literature cites conjugates, such as Poly(lactic acid)-poly(ethylene glycol) conjugates, as appropriate carriers for BMPs (Miyamoto *et al.*, Clin. Orthop. Rel. Res. 294:333 (1993)). Also useful as the carriers are fibrin-based polymers, in liquid or gel
15 form. Among the materials useful as sequestering agents are hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol), and cellulosic materials, such as hydroxycelluloses. One such preferred agent is carboxymethylcellulose.

20 The above materials disclosed to be useful as sequestering agents may themselves be useful as carriers for injection. In addition, combinations of the above described materials may be used.

It is also possible to use as injectable carriers solid materials which are narrow enough to be administered through injection. In such cases, the solid material may be combined with a viscous liquid carrier for ease and uniformity of administration.

25 In cases where the carrier may be of higher viscosity than optimal, the carrier may optionally be combined with a diluent, such as aqueous glycerol, preferably the carrier diluent would be present in concentrations of about 10 to about 80% (v/v). Also, the above materials may be combined in particular embodiments of the present invention. For example, polymers, such as porous particulate polymers, may be dissolved or suspended in cellulosic or gel carriers to increase viscosity.

30 In a preferred embodiment of the present invention, the active agents are administered locally through injection using only a suitable buffer as carrier. One suitable buffer comprises glycine, sucrose, and glutamic acid hydrochloride, at a pH of less than 6.0. Preferred compositions of buffer solutions comprise about 1.0 to about 10.0% (w/v) glycine, about 0.1 to about 5.0% (w/v) of a sugar, preferably
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5 sucrose, about 1 to about 20 mM glutamine, glutamic acid, or glutamic acid hydrochloride, and optionally about 0.01 to about 0.1% of a non-ionic surfactant, such as polysorbate 80. In a preferred embodiment of the invention, this formulation comprises about 2.5% glycine (g/100 ml (w/v)), about 0.5% sucrose (w/v), about 5 mM glutamic acid hydrochloride (about 0.1% w/v), and about 0.01% (w/v) polysorbate 80, at a pH of about 4.5. This buffer has been described as MFR 842. 10 Further buffers suitable for use in the present invention are described in United State Patent 5,385,887, the disclosure of which is hereby incorporated by reference. Preferred solutions may also include combinations of buffer and other carrier, such as a combination of buffer and cellulosic carrier. Preferred ranges for this combination 15 are from about 1% to about 20% w/v cellulosic carrier/buffer. If desired, a salt may be added.

MODE OF ADMINISTRATION

The injectable compositions of the present invention may be administered in any clinically acceptable manner of injection. A number of commercially available 20 syringes may be suitable for use in the present invention, and for administration of the compositions of the present invention. For example, suitable syringes are available the Calasept^R syringe [JS Dental Manufacturing, Ridgefield CT] comprises sterile calcium hydroxide paste in isotonic saline solution, in a non-aspirating or modified aspirating cartridge syringe; Henke-Ject Aspirating Syringe and Hypo Brand Dental 25 Syringes/Needles [Smith & Nephew MPL, Franklin Park, IL]; intraosseous needles from MPL, Inc., Chicago IL; and Luer-Lok^R Syringes [Becton Dickinson, Franklin Lakes, NJ], may all be appropriate syringes for use in the present invention.

In another embodiment of the present invention, bone morphogenetic proteins are used as an osteoinductive agent in the process known as distraction osteogenesis. 30 This process is an alternative to segmental bone regeneration in response to implanted osteoinductive agents. In traditional segmental bone repair, the osteoinductive agent and carrier are placed in the defect created between the parent bone ends. For bone formation to occur, the osteoinductive agent has to have sufficient residence time in the defect to stimulate differentiation of sufficient numbers of bone forming cells to 35 support new bone formation. The carrier also has to have sufficient residence time to

5 serve as a scaffold for bone forming cells to attach (osteoconduction). In addition, the carrier also has to have sufficient porosity to allow cells and blood vessels to penetrate into the defect for bone formation to occur. In contrast, the process of distraction osteogenesis creates a regenerate construct between the distracted parent bone ends that is highly vascular and contains a large population of mesenchymal stem cells
10 destined to become bone forming cells. As a result, the regenerate construct represents a much more ideal environment for cell differentiation growth factors such rhBMP-2 to stimulate rapid bone induction relative to induction of bone within a segmental defect.

The process of distraction osteogenesis begins with an initial latency period
15 allowing a fibrous connection to form between the bone ends to be distracted. Following this latency period, the bone ends are slowly distracted at a controlled rate of up to 1 mm per day in human clinical cases. Once the regenerate forms and the bone ends are distracted to the appropriate length, a prolonged consolidation period is required to allow the regenerate to form bone. This prolonged consolidation period
20 which can be on the order 4 to 6 months is associated with considerable morbidity. A frequent complication is the occurrence of pin track infections resulting from the extended length of time the external fixator used to generate the distraction must remain in place. In addition, there are considerable psychological effects and life style alterations associated with wearing the external fixator for prolonged periods of time.
25 In addition to complications associated with the external fixator, there are a number of patients where the regenerate does not form properly and a delayed union or non-union occurs. Since the regenerate contains a responsive cell population and is already highly vascularized following the initial distraction phase, the use of bone morphogenetic proteins may rapidly accelerate the rate of bone formation during the
30 normally prolonged consolidation phase of distraction osteogenesis. Acceleration of the distraction phase is limited by stretching of the soft tissues associated with bone. The cells created using distraction osteogenesis may also be harvested in order to provide a source of cells which are primed for osteogenesis. These cells can be cultured to prepare immortalized cell lines. If desired, these cells can also be
35 immunotolerized using agents such as CTLA4 receptors [U.S. Patent 5,434,131] or

5 CTLA4 ligands or B7 monoclonal antibodies [WO 96/40915]. Methods and materials for such immunotolerization are disclosed in the above references, and include co-transfection or treatment with these factors. The disclosure of these references is hereby incorporated herein by reference.

10 The following examples further describe the practice of embodiments of the invention with rhBMP-2 in a buffer carrier. The examples are not limiting, and as will be appreciated by those skilled in the art, can be varied in accordance with the above specification.

EXAMPLES

15 **Example 1: Oophorectomized Rat Model:**

The oophorectomized rat is a well-established model of osteoporosis and its use is recommended by the FDA as one of the animal models to be employed in demonstrating efficacy of potential therapeutic agents for osteoporosis. Preclinical studies testing the efficacy of rhBMP-2 in the oophorectomized rat model of osteoporosis demonstrated that 100 μ g rhBMP-2 in MFR 842 buffer, injected into the intramedullary space of the tibial shaft, was associated with extensive bone formation. Further evaluation with this model is conducted at lower doses.

20 In a similar study, five orariectomized rats were injected with 40 μ g of rhBMP-2 in MFR 842 buffer solution into one side of the femoral neck, and MFR 842 buffer alone on the other side. After six weeks, the femora are harvested, analyzed by DEXA for bone density and by histology.

25 In a larger study, six weeks after ovariectomy, rat tibiae are injected with 0, 10, or 50 μ g of rhBMP-2 in 20 μ L MFR 842 buffer. Controls include sham ovariectomized animals [surgery performed, but ovaries are not removed], buffer injections and sham surgeries. After six weeks, the femora are harvested and groups analyzed by DEXA, biomechanics, and histomorphometry.

30 **Example 2. Sheep Core Decompression Model**

35 Preclinical studies in support of the application of rhBMP-2 to the treatment of osteonecrosis of the hip at the time of core decompression demonstrated that

5 rhBMP-2 stimulates bone formation in the core decompression track within the femoral neck of normal (non-osteoporotic) sheep. In this study, defects in both the femoral head/neck and distal femur were created, the latter to facilitate DEXA imaging. rhBMP-2 in various formulations, including buffer, blood, blood clot and collagen dispersions was placed at the site of defect.

10 Example 3. Human Core Decompression Study.

Patients with osteonecrosis of the femoral head were treated by core decompression with or without implantation of blood clot containing rhBMP-2 into the core track. Patients who received rhBMP-2 experienced a reduction in the volume of necrotic bone compared to patients treated by core decompression alone. The treatment was well tolerated with complications limited to some bone formation adjacent to the cortical entry site.

15 Example 4. Biodistribution of rhBMP-2.

20 Four aged ewes underwent surgical placement of a cannulated needle into the femoral neck using uniplanar fluoroscopic guidance. A bolus injection of radiolabelled rhBMP-2 in buffer solution was delivered through the needle into the proximal femur. Local (bone) distribution of rhBMP-2 was monitored by gamma camera. Systemic distribution was assessed by periodic serum sampling. *In vivo* gamma camera images of the femur indicate that the injected rhBMP-2 enters the femoral head and neck and that part of the injected material is retained at these sites for at least 72 hours. Serum analysis of radiolabelled rhBMP-2 indicates that the majority (~85%) of the rhBMP-2 enters the systemic circulation within five minutes of administration.

25 Example 5. Intra-Femoral Injections.

30 Patients with osteoporosis who have sustained a fracture of the proximal femur are identified. Intraosseous administration of injectable rhBMP-2 is accomplished in the non-fractured (contralateral) femur using an operating room, biplanar fluoroscopy, and patient positioning on a fracture table. Each patient undergoes the necessary surgical treatment of the fractured femur. At the completion of surgical procedure, but before termination of the anesthesia, the lower extremities are repositioned on the fracture table to gain access to the uninjured contralateral hip. Under sterile

5 conditions and biplanar image intensifier visualization, the designated dose of rhBMP-2 is delivered by percutaneous, intraosseous route into the proximal femur. Follow-up evaluations include radiographic assessment, clinical evaluation of infection and fracture, and DEXA evidence of bone formation.

Example 6: Rabbit Ulnar Osteotomy and Fracture Models.

10 In the osteotomy model, the ulna is simply exposed, the periosteum is stripped, and a 2 to 5 mm osteotomy created using an oscillating saw. The incision is then closed, and the appendage bandaged to provide additional stability. Bilateral surgeries may be used in order to compare active agent against untreated or control substances.

The rabbit ulnar fracture model is similar to the rat femoral fracture model.

15 The rabbit ulnar fracture model relies on a weight-driven blunt instrument to create the fracture essentially using incident three-point bending. The fracture is created by fully extending and clamping the forelimb to an aluminum rod containing two vertical bars. In a modification from the rat apparatus, a 23G needle is placed in between the radius and ulna to serve as a guide pin. The blunt guillotine is then positioned, and 20 a 850 gm weight raised to 45 cm and allowed to fall to create the fracture.

25 Assessment of efficacy in both the rat femoral and rabbit ulnar fracture models can be accomplished primarily using biomechanics. Qualitative measures of fracture repair, such as radiology, can be obtained, but it is difficult to see subtle differences over normal fracture repair, and will not give a convincing demonstration that the injection is translating to accelerated fracture repair.

Example 7: Rat Ectopic Implant Models

In one experiment, several non-collagenous materials were tested in the rat ectopic implant model. The materials included in this study were:

1. MFR 842 buffer;
- 30 2. Poloxamer 407 gel.
3. Poly(lactide)/poly(ethylene glycol) gels. Low molecular weight (2000) PLA dissolved in PEG produces a syringeable solution that precipitates PLA upon injection into an aqueous environment, resulting in a relatively firm gel. Two different percentages were tested.
- 35 4. Poly(lactic acid)-poly(ethylene glycol) conjugates.

5 Each material was implanted with 0, 10, and 80 µg rhBMP-2. Lyophilized
rhBMP-2 was mixed with the gels using a mortar and pestle. The buffer, Pluronic
gels, and PEG/PLA gels were first injected into blood clots. The PLA-PEG
conjugates were implanted directly into the subcutaneous space. Six implants (three
10 animals) were used per group. In addition, the intramuscular injection technique was
investigated using the PLA/PEG gel and one Pluronic gel (both at the high dose only).

10 The above were scored for bone formation. Bone score is a semi-quantitative
measurement of the area of bone present in representative histological sections; the
score ranges from 0 for no bone present, to 5 when the entire section consists of bone.
Coupled with the wet weights and qualitative histological assessment of the sections,
15 the following conclusions can be made. The Pluronics produced relatively little bone
at the lower dose of rhBMP-2, and induced none when placed intramuscularly.
Presumably the material does not have a long enough residence time, or does not
sequester rhBMP-2 sufficiently. In addition, a significant inflammatory response was
noted. The PLA/PEG gels produced reasonable quantities of bone, with some residual
20 matrix observed (presumably the precipitated PLA) along with a foreign body giant
cell response. The PLA-PEG conjugates produced well-defined ossicles of bone with
bone marrow. Of the gels tested, this material performed best. Based on the samples
tested, the intramuscular implantation appeared to be a more rigorous test of materials.
In this experiment, rhBMP-2 in buffer simply injected into blood clot performed the
25 best overall; presumably the rhBMP-2 is able to rapidly distribute and bind to the
blood clot.

25 In another rat ectopic study, several cross-linked and non-cross-linked collagen
materials were tested. In addition, porosity was introduced into the PLA/PEG gels by
the addition of MFR 842 buffer as an excipient, and a lower percentage of PLA was
30 tested. Some of the collagen materials were tested in an aqueous (MFR 842) or non-
aqueous (PLA/PEG gel) environment. The collagen materials tested included:

- 35 1. Homogenized Helistat® bovine type I collagen cross-linked hemostatic
sponge (currently in use with rhBMP-2 clinically) made into a suspension;
2. Homogenized Helitec™ (fibrillar form). Only tested with PLA/PEG in this
experiment;

5 3. Collagen dispersion (Integra LifeSciences, Plainsboro, NJ). The non-cross-linked parent material of Helistat®. At the concentration tested (0.78%, as provided), it is not viscous at all.

4. Zimmer collagen gel. The collagen component of the Collagraft® kit.

10 All materials were tested with 10 µg rhBMP-2 using the blood clot injection method. The samples were scored for histologic bone formation. The materials which performed best in this study were the Helistat® dispersion and the collagen gel, yielding bone scores of 3 to 4, and no adverse tissue reaction. All PLA/PEG containing materials showed much smaller amounts of bone, and significant inflammatory response (non-specific foreign body type). Addition of PLA/PEG to the 15 collagen material decreased the bone formation observed. In this experiment, the rhBMP-2 in buffer placed in blood clot performed poorly; this may be because a larger gauge needle was used to place the material, and it flowed back along the needle track before it could distribute in the blood clot. Likewise, the ILS dispersion performed poorly, perhaps due to low viscosity. These results indicate relatively little promise 20 for the PLA/PEG gels, unless use of very low molecular weight PLA accelerates its resorption time. The collagen materials stand out above the other materials tested to date using this rat ectopic assay system.

Various collagen materials tested include 2% ILS dispersion, 2% Hilitene™ dispersion, 2% Helistat® dispersion, the Zimmer collagen gel, and another collagen 25 gel (6.5%) from Matrix Pharmaceuticals. All have been placed at 0, 20, and 80 µg rhBMP-2 and analyzed histologically.

Example 8: Distraction Osteogenesis

BMP-2 may accelerate the consolidation (bone formation or mineralization) 30 phase in a rabbit model of limb lengthening. Osteotomies are created in rabbit tibia. The tibia are then be distracted 2 cm over a period of approximately ten days. Following the distraction phase, rhBMP-2 is administered. Two modes of application 35 are tested. One set of animals has rhBMP-2/ACS (1.5 mg/mL; 0.075 mg/tibia) surgically placed within the regenerate. The second group of animals has rhBMP-2/MFR842 buffer (0.75 mg/mL; 0.075 mg/tibia) percutaneously injected into

5 three sites within the regenerate. A third group of control animals has surgical intervention similar to the rhBMP-2/ACS-treated animals, with placement of ACS/buffer within the regenerate. Additional sets of animals have distraction performed, with no surgical intervention. Groups of animals are sacrificed at 5, 14 and 24 days. Radiographs are taken following the distraction phase and at sacrifice
10 and are used to evaluate new bone formation in response to treatment and control. Bone density within the defect is determined with Dual Energy X-Ray Absorptiometry and Peripheral Quantitative Computed Tomography (pQCT). The three dimensional distribution of bone within the defect is evaluated with pQCT. Histological evaluation of non-decalcified specimens is used to quantify histomorphometric parameters.

15 Results to date indicate a visually detectable acceleration of bone induction in rhBMP-2/ACS treated animals compared to untreated surgical controls at 5 days and 14 days based on radiographic evaluation. The DXA and pQCT images confirm these findings. In addition the pQCT images indicate greater three-dimensional distribution of bone within the regenerate and evidence of early stages of corticalization in the
20 rhBMP-2/ACS treated animals at 14 days. Further results may support the rhBMP-2/ACS treatment effect of significant acceleration of the consolidation phase of distraction osteogenesis. Formation of an intact cortex is the primary indicator for removal of the external fixator and associated return to unprotected full weight bearing. The rhBMP-2/MFR842 injection buffer treatment is the preferred route of
25 administration having more wide spread application in primary distraction osteogenesis.

CLAIMS

We claim:

1. A method for reducing the severity and/or incidence of fractures comprising administering to a site of osteoporotic or osteopenic bone tissue an injectable composition comprising an effective amount of an active agent in a suitable carrier.
- 10 2. The method of claim 1, wherein the active agent is a member of the TGF- β superfamily of proteins.
3. The method of claim 1, wherein the active agent is a member of the bone morphogenetic protein family of proteins.
- 15 4. The method of claim 1, wherein the active agent is selected from the group consisting of BMP-2, BMP-4, BMP-5, BMP-6, BMP-7, BMP-10, BMP-12 and BMP-13.
5. The method of claim 1, wherein the active agent is BMP-2.
6. The method of claim 1, wherein the carrier is selected from the group consisting of a suitable buffer, blood, collagen gel and injectable calcium phosphates.
- 20 7. A method for healing bone fractures comprising implanting BMP into the region between ends of the bone fracture and allowing such rhBMP-2 to stimulate bone induction.
8. A cell produced by the method of claim 7 and harvested from the region between bone ends.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/01143

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12N5/08 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	US 5 597 897 A (RON EYAL ET AL) 28 January 1997 * see in particular examples 4,5 and col. 5, 1. 58- col.6, 1.7 * & WO 93 00050 A (GENETICS INST.) 7 January 1993 ---	1-7
X	US 5 453 419 A (MURAKAMI KAZUO ET AL) 26 September 1995 *see in particular col. 7, 1.63 - col. 8, 1. 12, and claims 1-2*	1-7
X	US 5 166 058 A (WANG ELIZABETH A ET AL) 24 November 1992 *see in particular col.5 1. 50 - col.7, 1.43 * ---	1-7
	-/-	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/01143

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WANG: "Bone morphogenetic proteins (BMPS): Therapeutic potential in healing bony defects" TRENDS IN BIOTECHNOLOGY, vol. 11, no. 9, 1993, pages 379-383, XP002065499 * see in particular p.381, 2nd paragraph -382 *</p> <p>---</p>	1-7
X	<p>REDDI ET AL.: "Osteoporosis (chapter 9, p. 281-287)" 1996, ACADEMIC, SAN DIEGO, CALIF. XP002065501 *see in particular p. 285 *</p> <p>---</p>	1-7
X	<p>TAGAKI ET AL.: "The role of bone marrow in bone morphogenetic protein-induced repair of femoral massive diaphyseal defects" CLINICAL ORTHOPAEDICS AND RELATED RESEARCH, vol. 171, 1982, pages 224-231, XP002065500 * see in particular p. 230 (summary) and Figures 1-5 n*</p> <p>-----</p>	1-3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/01143

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: 8 because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Claim 8 relates to a cell "produced by the method of claim 7". However, claim 7 does not describe such a method but relates to a method of treatment.

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/01143

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5597897	A 28-01-1997	AU 663328 B DE 69213739 D DE 69213739 T EP 0591392 A FI 935732 A JP 6508777 T NO 934573 A AT 142460 T AU 2254292 A CA 2111199 A ES 2094359 T MX 9203083 A WO 9300050 A	05-10-1995 17-10-1996 20-02-1997 13-04-1994 20-12-1993 06-10-1994 13-12-1993 15-09-1996 25-01-1993 07-01-1993 16-01-1997 31-08-1994 07-01-1993
US 5453419	A 26-09-1995	US 5670338 A AT 140965 T CA 2024629 A DE 69027961 D DE 69027961 T EP 0416578 A JP 4154799 A	23-09-1997 15-08-1996 07-03-1991 05-09-1996 09-01-1997 13-03-1991 27-05-1992
US 5166058	A 24-11-1992	US 5013649 A US 5631142 A AT 141928 T AU 613314 B AU 7783587 A DE 3751887 D DE 3751887 T DK 53497 A DK 106288 A EP 0313578 A EP 0688869 A IE 75881 B IL 83003 A JP 2729222 B JP 6298800 A JP 10070989 A JP 2500241 T JP 2713715 B	07-05-1991 20-05-1997 15-09-1996 01-08-1991 29-01-1988 02-10-1996 06-03-1997 09-05-1997 28-04-1988 03-05-1989 27-12-1995 24-09-1997 31-07-1995 18-03-1998 25-10-1994 17-03-1998 01-02-1990 16-02-1998

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/01143

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5166058	A	KR 9705583 B	18-04-1997
		MX 170919 B	22-09-1993
		PT 85225 B	30-03-1990
		WO 8800205 A	14-01-1988
		US 5543394 A	06-08-1996
		US 5459047 A	17-10-1995
		US 5635373 A	03-06-1997
		US 5187076 A	16-02-1993
		US 5618924 A	08-04-1997
		US 5116738 A	26-05-1992
		US 5366875 A	22-11-1994
		NO 963788 A	17-02-1988
		NO 963789 A	17-02-1988
		US 5106748 A	21-04-1992
		US 5141905 A	25-08-1992
		US 5108922 A	28-04-1992